THE LOCATION OF CONTACT CHEMORECEPTORS SENSITIVE TO SUCROSE SOLUTIONS IN ADULT TRICHOPTERA [1]

HUBERT FRINGS AND MABLE FRINGS

Department of Zoology and Entomology, The Pennsylvania State University, University Park, Pennsylvania, and Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine

Descriptions of the mouth-parts and feeding of adult Trichoptera in recent American text books and other general works on entomology are not consistent. Folsom and Wardle (1934, pp. 20, 39), Frost (1942, p. 89), Comstock, (1950, p. 555), Metcalf, Flint and Metcalf (1951, p. 229) and Ross (1948, p. 367) described the mouth-parts as "vestigial," "rudimentary," "subatrophied," or "greatly reduced." These views carried as a corollary the belief that the adults take little or no nourishment, and Brues (1946, p. 44) listed caddis-flies with the "aphagia": insects "which do not feed at all after maturity." Borror and DeLong (1955, p. 437), however, described the mouth-parts as "chewing type, with the palpi well developed but with the mandibles much reduced," and stated that "the adults feed principally on liquid foods." Swain (1948, p. 79) also stated that adult caddis-flies take liquid food, but termed the mouth-parts a "short, uncoiled proboscis."

A review of earlier accounts reveals a similar lack of agreement. Réaumur (1737, pp. 175–176) wrote that the mouth-parts of Trichoptera are for sucking and lapping, like those of Diptera. Kirby and Spence (1826, p. 464, Pl. VII, Fig. 1), on the other hand, regarded the mouth-parts as modified mandibulate. Burmeister (1832, pp. 68; 377–378) stated that the mouth-parts are intermediate between the mandibulate and haustellate types, comparing them with those of bees. Lucas (1893) made a detailed study of the mouth-parts of *Anabolia furcata* (= *laevis*). He found the mandibles to be atrophied, the labrum and maxillae reduced, and the labium developed into a sucking organ, the haustellum. Ulmer (1904) and Cummings (1913, 1914) reported a well developed haustellum to be present in every family of Trichoptera. These facts are reported in the special works on Trichoptera by Betten (1934, pp. 19–22), Ross (1944, p. 4) and Mosely and Kimmins (1953, pp. 10–11), and in the text books of Packard (1898, pp. 74–75), Weber (1933, pp. 66–68; 1954, pp. 297–298), and Imms (1948, pp. 19, 411–412).

Réaumur (1737, pp. 175–176) stated that adult Trichoptera take liquid foods, and Burmeister (1832, pp. 377–378) reaffirmed this, reporting that he found them feeding on nectar of flowers. Lucas (1893) reported finding tiny particles like pollen in the folds of the haustellum, and he therefore believed that they feed on nectar. There were other workers who made observations, often quite casual, that confirmed or contradicted these ideas. These are reviewed in the papers of Siltala (1907) and Döhler (1914). Siltala (1907) gave adult *Phryganea striata* and *Limnephilus rhombicus* only water for three or four days, then placed a flowering

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branch of *Spirea* near them. They flew to the flowers and fed on the nectar, thus confirming the reports of feeding by adults.

Döhler (1914) made many observations that leave little doubt that adult Trichoptera feed. He fed them on sucrose solution to which litmus was added and followed the changes in acidity in the gut as an indication of digestion. He fed ferric lactate in water and demonstrated the absorption of iron by the gut-wall. When he gave only water to 23 individuals of *Limnephilus flavicornis*, they survived for 19–40 days. When he gave sugar-water to 19 others, they lived 45–105 days. He observed feeding closely by seizing the wings of the insects and holding them as he brought drops of sugar-water to their mouth-parts. He found, in *L. flavicornis*, such greedy acceptance of the food that some individuals ruptured the intestine through over-feeding. All these laboratory observations led Döhler to conclude that adult Trichoptera feed in nature, and he supported this by reference to Siltala's observations and those of earlier workers who reported finding caddis-flies on flowers or at sweet baits used to lure moths.

Lucas, Siltala and Döhler, thus, seem to have shown convincingly that the mouth-parts of some larger adult Trichoptera are functional. The labrum is reduced, the mandibles rudimentary, the maxillae modified, the maxillary and labial palpi well developed, and the hypopharynx or labium or both developed into an extensible haustellum. These species of Trichoptera, at least, probably feed in nature on nectar and other sweet substances.

The present paper reports experiments on four species of Trichoptera from two families, and observations on two other species from two more families. The purpose of the experiments was to discover the location of the contact chemoreceptors mediating feeding responses when stimulated with sucrose solution. The results further support the belief that the mouth-parts of adult Trichoptera are functional and that the insects feed in the adult stage.

Materials and Methods

The following species of Trichoptera were studied experimentally. Identifications were made by the authors with the aid of works of Betten (1934), Milne (1934–36) and Ross (1944). Dr. H. H. Ross kindly checked the identifications, and we wish to thank him for this.

Family: Phryganeidae
- *Banksiola smithi*—2 males, 4 females
- *Ptilostomis ocellifera*—1 male, 6 females
- *Phryganea sayi*—4 males, 8 females

Family: Limnephilidae
- *Platycentropus radiatus*—8 males, 9 females

All the experimental subjects were captured when they came to lights at night. They were lightly anaesthetized with ether and mounted alive by fastening the dorsal side of the thorax and the wings to a wax block on the end of a glass rod (Fig. 1). These are relatively large (*B. smithi* about 15 mm. long; the others 20–25 mm. long), and when they were thus mounted could easily be observed. Longevity was good if the animals were fed and watered daily; even with legs and other parts removed they lived for up to 26 days.

The contact chemoreceptors were located by the methods described in detail in Frings and Frings (1949). The animals were tested daily after night fall, for they responded more actively at night, even with the necessary artificial illumination, than in day light, as Döhler (1914) also noted. Before each daily series of tests, the animals were given water to satiety. It was essential that water-satiety be maintained in the subjects, because the tests involved discrimination between water and water with sucrose added. When the animals had taken all the water they would take, water was brought to the locus being tested on an artist's brush or glass mi-

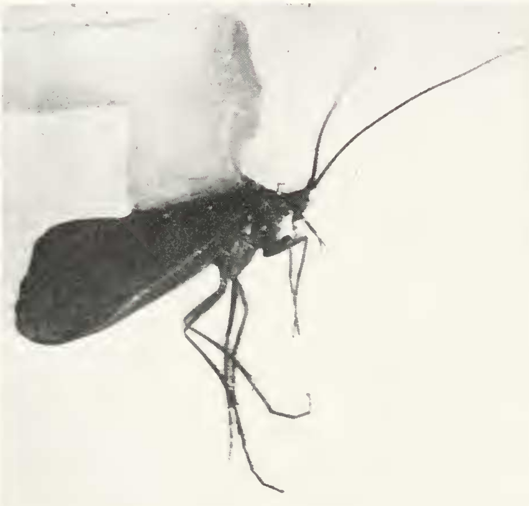

FIGURE 1. A caddis-fly (*Platycentropus radiatus*) mounted alive on a paraffin block on the end of a glass rod ($3\times$).

croneedle and the reaction noted under a binocular dissecting microscope. This was followed by a similar trial with 1 *M* sucrose solution, and the reaction again noted. These presentations were repeated a sufficient number of times to be sure that the insect responded similarly or differently to the two stimuli. Such a series of trials constituted one test. There were variable numbers of tests carried out at each testing period. At the end of each daily testing period, the insects were fed to satiety on the sucrose solution. They imbibed heavily, but did not damage themselves, as Döhler reported for the animals he tested.

When preliminary experiments had revealed possible loci of contact chemoreceptors, the structures were removed and the animals retested similarly. Operations were performed under light anaesthesia with paired controls anaesthetized and sham operated. For microscopic examination of possible end-organs on the experimentally determined loci, the structures were removed in 70% ethyl alcohol, transferred to 95% and thence to Diaphane on micro slides.

The following species were observed unmounted:

Family: Leptoceridae
Oecetis cinerascens
Family: Hydroptilidae
Orthotrichia americana

Attempts to mount and test about 30 individuals of the first-named were made, but these did not survive more than one day. Both of these species were very common and, as described by Döhler, highly attracted to sugar-water. They came onto the laboratory table and could be given water and sucrose solutions while they scurried about. By carefully controlling the placement of droplets near them, it was possible to test them and to observe feeding. *O. cinerascens* is large enough (about 12 mm. long) to be observed with the naked eye. *O. americana,* like all Hydroptilidae, is quite small (about 3 mm. long). It was necessary, therefore, to observe it with a dissecting microscope. Luckily the insects came right onto the brushes and needles used in testing the larger forms, and they were thus easily observed.

Results

With *B. smithi* only gross localization tests were made, using brushes with water and 1 *M* sucrose solution applied to various organs of the intact, mounted animals. The palpal tips proved to be quite sensitive: touching them with sugar-water brought about extension of the haustellum. The tarsi, likewise, had contact chemoreceptors: touching them with a brush bearing sugar-water induced eager reaching with the palpi toward the brush. With water alone on a brush, in each case, withdrawal or neutral reactions were elicited. Touching the antennae with water or sugar-water resulted in withdrawal of the antennae, indicating that these lack contact chemoreceptors sensitive to sucrose.

With *Pt. ocellifera, P. sayi* and *P. radiatus* more detailed experiments were carried out: with *Pt. ocellifera* about 100 tests were made over a period of 10–26 days; with *P. sayi*, about 200 tests over 16 days; with *P. radiatus* about 300 sets of tests over 16 days. These three species reacted almost exactly alike, and the results are thus given together. Figure 2 is a photograph of the head and mouth-parts of *P. radiatus* to show the well developed haustellum and palpi.

The first series of experiments was designed to give the general locations of the contact chemoreceptors. Using brushes in paired trials with water and 1 *M* sucrose solution, no evidence of discrimination was found when the antennae were tested. If the insects were "thirsty," however, the antennae were quite sensitive to water vapor. If a brush bearing water was brought near to but not in contact with the antennae, the insects almost immediately began to reach excitedly with the palpi. Once sated with water, however, this ceased, and the only reaction to contact with a brush moistened with water or sugar-water was withdrawal of the antennae. The conclusion that the antennae lack contact chemoreceptors, however, must be stated cautiously, for recent experiments with some Lepidoptera (Frings and Frings, 1956) show that reactions mediated by the antennae may depend upon presence or absence of contact chemoreceptors on other parts of the body.

Contact of the tarsi of water-sated individuals with sugar-water elicited reaching with the maxillary and labial palpi and partial extension of the haustellum. Ordinarily the palpi were folded against the head, and this reaction was quite clear-cut. Touching only the ventral surface of the tarsus of one fore leg with the brush mediated the same response. It was impossible, however, to touch the other tarsi in

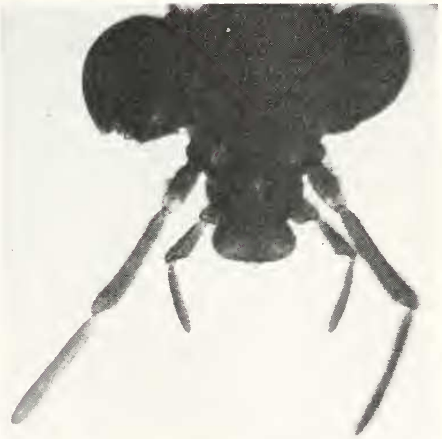

FIGURE 2. Head and mouth-parts of an adult caddis-fly (*P. radiatus*) showing the well developed maxillary and labial palpi and the medial haustellum ($25\times$).

an intact animal without having the fore tarsi also brought to the brush. The palpi likewise proved to be receptive: touching them with sugar-water elicited spreading of the haustellum. If 1 *M* NaCl solution was used instead of sucrose on the tarsi, there was no reaching with the palpi, and if it was used on the palpi, they were withdrawn sharply.

Further experiments on intact animals were made with glass micro-needles bearing water and sugar-water. The results with the antennae and tarsi were the same as when brushes were used. Touching only the tips of the maxillary palpi with sugar-water elicited reaching with both sets of palpi and partial spreading of the haustellum, much like the reaction obtained by touching the tarsi. Bringing sucrose solution to any part of a maxillary palpus other than the tip of the terminal segment brought about withdrawal, just as with water. Touching the maxillary palpal tips with NaCl solution elicited a sharp retraction of the palpi. Touching the tips of the labial palpi with sugar-water on a needle elicited spreading of the haustellum in preparation for feeding. Other parts of the labial palpi seemed not to be sensitive, as with the maxillary palpi. With NaCl solution, these palpi were also drawn away. The feeding reaction, therefore, seems to occur in two stages: 1) exploration with the palpi when an acceptable solution touches the tarsi or maxillary palpal tips, and 2) extension and spreading of the haustellum when the solution touches the tips of the labial palpi.

Following these tests, operations were performed to enable us to test parts that could not be touched without interference by known receptors. The forelegs were removed first. Using the brushes, the middle and hind tarsi together were found to be sensitive to sucrose. With care the middle tarsi together or singly could be touched, and this elicited the usual response. With the fore and hind legs removed, the middle tarsi were easily tested and found to bear contact chemoreceptors sensitive to sucrose. With the fore and middle legs removed, the hind tarsi together or singly were also found to be sensitive. All the tarsi, thus, bear the receptors. Using microneedles, the receptors were found on the ventral surfaces of the tarsi and not on the other parts of the legs, but the exact segments of the tarsi bearing them were not determined.

With the last segments of the maxillary and labial palpi removed, the reactions to contact of the palpi with sucrose solutions were abolished, thus confirming previous observations that the receptors were confined to these segments. To test the sensitivity of the haustellum, the fore and middle legs and the palpi were removed, and the animals offered water and sugar-water on brushes. A little difficulty was encountered at first, because the haustellum became covered with clotted hemolymph from the cut ends of the palpi. After this was washed off, however, the animals were able to feed. They obviously could distinguish sucrose solution from water, spreading the haustellum and drinking the former when sated with water. If NaCl was used instead of sucrose, they refused to drink. If they were drinking sugar-water and NaCl solution was suddenly substituted, they reacted by immediate withdrawal of the haustellum and often by violent retraction of the head. Thus the receptors could distinguish acceptable from unacceptable materials in solution. The receptors were not located exactly on the haustellum, but they would seem to be near the distal margins, for application to the tip of the haustellum of a droplet of sugar-water on a needle brought about almost immediate extension.

The parts of the body bearing contact chemoreceptors sensitive at least to sucrose and NaCl, therefore, are the ventral sides of all the tarsi, the tips of the terminal segments of the maxillary and labial palpi and the haustellum. Generally the reaction to appropriate stimulation of the tarsal or maxillary palpal receptors is reaching with and vibration of the palpi and partial extension of the haustellum. The reaction to appropriate stimulation of the labial palpal tips or the haustellum is extension and spreading of the haustellum and feeding. No differences were noted between the reactions of males and females.

The tarsi, palpi and haustellum of these three species were mounted on slides and examined in an attempt to find the possible end-organs involved. On the ventral surfaces of the tarsi there are many short, thin walled, trichoid sensilla among the longer hairs and spines. These are quite similar to the probable receptors in

Lepidoptera and Diptera (Eltringham, 1933; Frings and Frings, 1949; Grabowski and Dethier, 1954; Hayes and Liu, 1947; Lewis, 1954a, 1954b; Tinbergen 1939). On the palpi there are many trichoid sensilla on all the segments, but there is no way at present to select any as possible receptors. On the posterior face of the haustellum, there are basiconic and trichoid sensilla in small numbers. Either of these might be involved, because no other obvious sensilla are present, but the data do not warrant any definite selection. It is probable, therefore, that the receptors on the tarsi and palpi are trichoid sensilla, while those on the haustellum are either trichoid or basiconic.

With *O. cinerascens* only a few tests were made on mounted animals before their untimely deaths. It was obvious that they had tarsal and palpal receptors like the others tested, but these were not further localized. With this species and with *O. americana* many observations were made on unmounted individuals that visited the laboratory table where the others were being tested or came onto the brushes and needles used in the experiments. They scurried about in characteristic, excited manner, turning to and fro, antennae vibrating. If a droplet of water was in their way, they usually stopped as soon as they touched it and drank. When sated with water, they no longer stopped at these droplets. If a droplet of sucrose solution was placed in their way, however, they stopped as soon as the tarsi touched it and turned round and round reaching with the palpi. As soon as the palpi touched the droplet of sugar-water the haustellum was extended and the insect fed.

Discussion

These observations and experiments on adults of six species of Trichoptera representing four families show that, in these at least, the mouth-parts are functional, modified for sucking, and that the adults feed. These results are fully concordant with the reports of Lucas (1893), Siltala (1907) and Döhler (1914). Ulmer (1904) and Cummings (1913, 1914) reported that only a few species from one or two families lack a well developed haustellum. While all the observations on living animals have been made on representatives from only four families and mostly from two families, the conclusions may have validity for Trichoptera generally. At least no one has shown experimentally that any species does not feed in the adult state.

The presence of tarsal contact chemoreceptors also indicates affinity with the haustellate insects. Those studied to date (Hemiptera, Lepidoptera, Diptera, Hymenoptera), in contrast with the mandibulate forms, have contact chemoreceptors on the tarsi (Frings and Frings, 1949). The near certainty that the tarsal and palpal end-organs of Trichoptera are trichoid sensilla further allies them with the typical haustellate forms. Haustellate species, in general, have trichoid sensilla for trophic contact chemoreception and mandibulate forms basiconic sensilla (Frings and Frings, 1949). In the presence of contact chemoreceptors on the palpi and the absence on the antennae, the Trichoptera are more like the Diptera than the Lepidoptera (Frings and Frings, 1949, 1956). The general form of the mouth-parts in Trichoptera also is more like that of Diptera than Lepidoptera. How much weight can be attached to evidence such as this in determining relationships among larger groups of insects we do not know. Certainly, however, further comparative studies of contact chemoreception in Trichoptera, as well as other haustellate groups, would be desirable.

Summary

The loci of contact chemoreceptors sensitive to sucrose and NaCl in solution and mediating feeding responses were determined experimentally in adult Trichoptera of four species from the families, Phryganeidae and Limnephilidae. The receptors are on the ventral surfaces of all the tarsi, the tips of the maxillary and labial palpi and the haustellum. The animals feed on liquids, and these receptors allow them to distinguish acceptable from non-acceptable materials in solution. Less precise observations on two other species from two other families showed a similar situation in these. The end-organs are probably trichoid sensilla. This fact, along with the presence of tarsal contact chemoreceptors, places adult Trichoptera of these species, at least, among typical haustellate insects, most nearly resembling many Diptera in locations of the receptors and feeding reactions.

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